

# Effect of Gibberellic Acid and Dry Yeast on Growth, Yield, and Essential Oil of Lemon Balm (*Melissa officinalis* L.)

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## ABSTRACT

In a field experiment during two successive seasons (2005-2006 and 2006-2007), the effect of gibberellic acid (GA<sub>3</sub>) and active dry yeast on growth, yield, and essential oil (EO) of lemon balm plants was investigated. Application of GA<sub>3</sub> and/or active dry yeast increased vegetative characters (i.e. plant height, number of branches, and herb fresh and dry weight per plant) compared to control (sprayed with water only). The maximum mean values of growth characters were obtained as a result of spraying with 6 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>. The lowest fresh and dry weight of plants were observed with the treatment of 2 g  $\Gamma^1$  yeast + 0 ppm GA<sub>3</sub> in the first harvest. EO content in the lemon balm herb increased due to the application of GA<sub>3</sub> and/or active dry yeast compared to control. The highest EO yield per plant was observed with the treatment of 6 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>. The lowest amount of EO yield was obtained with the control treatment. The highest geranial in lemon balm EO occurred with the treatment of 6 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>.

Keywords: active dry yeast, antioxidant, GA<sub>3</sub>, geranial, neral

## INTRODUCTION

Lemon balm (Melissa officinalis L.; Fam. Lamiaceae) is one of the most important medicinal and aromatic plants due to its culinary properties and grows widely in central and southern Europe and slightly in Asia (Zargari 1990). It is used in conventional medicine for the treatment of headaches, flatulence, indigestion, colic, nausea, nervousness, anemia, vertigo, syncope, malaise, asthma, bronchitis, amenorrhea, cardiac failure, arrhythmias, insomnia, epilepsy, depression, psychosis, hysteria, ulcers and wounds (Zargari 1990; Anon 2002). Recently, several investigators have studied the antioxidative activity of lemon balm (Hohman et al. 1999; Triantaphyllou et al. 2001; Ivanova et al. 2005; Venkutonis et al. 2005; Dastmalchi et al. 2008). The application of phytohormones, gibberellic acid (GA<sub>3</sub>) and/or the unicellular fungi producing auxin, yeast showed different effects on different plants, e.g., GA3 application on Tagetes patula at 100, 200 or 400 ppm increased the plant height, fresh and dry weight per plant, but had no effect on the number of branches (El-Leithy 1987).

The role of gibberellins has been indicated many years ago, Leopold and Kriedeman (1975) emphasized that GA<sub>3</sub> promotes growth by its effect on cell division and cell enlargement. The influence of GA<sub>3</sub> on the plant height was proved through the studies of El-Keltawi (1981) on Mentha viridis; Hamza et al. (1981) on Pelargonium ortorum; El-Leithy (1983) on Ocimum basilicum; Salem (1984) on Chrysanthimum sp.; and Al-Mulla (1985) on Tagetes erecta. Mazhar (1988) reported that GA<sub>3</sub> treatments at 50 and 100 ppm increased the plant height, number of branches, total umbels per plant of Ammi visnaga plants. Sharma et al. (1988) on *Mentha citrata* indicated that foliar spraying with 100, 200 or 400 ppm of GA<sub>3</sub> increased plant height, number of leaves, fresh and dry weight per plant. Roselle (Hibiscus sabdariffa) plants sprayed with dry yeast at a rate of 2 g l<sup>-1</sup> showed the highest yield of calyxes (Ahmed *et al.* 1998). Khedr and Farid (2000) demonstrated that the effect of dry yeast is due to its effect on induction of endogenous hormones like  $GA_3$  and IAA. Vitamins are known as growth factors influencing physiological process (Chailakhyan 1957). Recently, dry yeast has used as an alternative source to growth substances in bio/organic fertilization system (Khedr and Farid 2000).

The objective of this study was to investigate the growth, yield, and chemical composition of lemon balm plants as influenced by the application of gibberellic acid  $(GA_3)$  and/ or active dry yeast.

### MATERIALS AND METHODS

Seeds of lemon balm (Melissa officinalis L.; Fam. Lamiaceae) were supplied by Jelitto GmbH, Germany. Two field experiments were carried out during two successive seasons (2005-2006 and 2006-2007) at the National Research Centre (NRC) Experimental Station (30° 05' N, 31° 22' E), Al-Giza Governorate, to study the effect of GA3 in combination with active dry yeast (Saccharomyces cerevisiae) on growth and EO production of lemon balm plants. The main weather information for Cairo, Egypt concerning temperature (T), sunshine (SH) and rainfall (RF) is given in Table 1. The physical and chemical analyses of the field experiment soil (Table 2) were carried out before planting according to the method of Chapman and Pratt (1978). Ninety kg P2O5/ha (calcium superphosphate 15.5% P2O5) were applied during ploughing. Plants were side-dressed with 200 kg N/ha (ammonium sulphate 20.5% N) and 65 kg K<sub>2</sub>O/ha (potassium sulphate 49% K<sub>2</sub>O) in two doses: first dose 30 days after transplanting, second 90 days after transplanting; after first harvest. Furrow irrigation was applied as required. The powder form of the active dry yeast was purchased from the local market. Yeast was activated overnight by sucrose at a rate of 2 g l<sup>-1</sup> aqueous solution of dry yeast before spraying on the plants. The plants were sprayed drop wet. The GA<sub>3</sub> used was in commercial form of Berelex (gibberellin C19H22O6 contains 90% GA<sub>3</sub>) purchased from the local market.

Treatments (T) used in the field experiments were identified as follows: T1 (0 g  $\Gamma^1$  yeast + 0 ppm GA<sub>3</sub>; control sprayed with water), T2 (0 g  $\Gamma^1$  yeast + 100 ppm GA<sub>3</sub>), T3 (0 g  $\Gamma^1$  yeast + 200 ppm GA<sub>3</sub>), T4 (0 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>), T5 (2 g  $\Gamma^1$  yeast + 0

Table 1 Average values of main weather variables in Cairo\*.

| Feature | Jan. | Feb. | Mar. | Apr. | May  | Jun. | Jul. | Aug. | Sept. | Oct. | Nov. | Dec. |                   |
|---------|------|------|------|------|------|------|------|------|-------|------|------|------|-------------------|
| T (°C)  | 13.5 | 14.9 | 17.5 | 21.0 | 24.5 | 27.1 | 27.7 | 27.6 | 25.9  | 23.4 | 19.1 | 15.1 | 21.4 <sup>1</sup> |
| SH (h)  | 213  | 222  | 243  | 279  | 315  | 372  | 357  | 333  | 309   | 297  | 228  | 201  | 3369 <sup>2</sup> |
| RF (mm) | 5.2  | 3.5  | 2.4  | 1.1  | 0.6  | 0.1  | 0.0  | 0.0  | 0.0   | 1.0  | 3.4  | 6.6  | $23.9^{2}$        |

**Table 2** Properties of the soil used for growing lemon balm.

| 14.04 20.75 55 |      |      |      |     |     |      |      |
|----------------|------|------|------|-----|-----|------|------|
| 14.94 29.75 55 | 0.16 | 0.23 | 8.23 | 2.8 | 480 | 37.8 | 35.7 |

<sup>1</sup> – organic matter, <sup>2</sup> – total, <sup>3</sup> – available.

Table 3 Effect of GA<sub>3</sub> and dry yeast on growth, yield and essential oil percentage of lemon balm during the first season.

| Treatments          | Gibberellic                  | Plant h | eight (cm) | Numbe    | r of  | Plant fr | esh        | Plant dr | y weight |      | lry weight           | Essenti | al oil (%   |  |
|---------------------|------------------------------|---------|------------|----------|-------|----------|------------|----------|----------|------|----------------------|---------|-------------|--|
|                     | acid (GA <sub>3</sub>        |         |            | branches |       | weight ( | weight (g) |          | (g)      |      | ha <sup>-1</sup> (t) |         | dry weight) |  |
|                     | in ppm)                      | I*      | II**       | I*       | II**  | I*       | II**       | I*       | II**     | I*   | II**                 | I*      | II**        |  |
| Yeast               | 0                            | 38.21   | 32.88      | 5        | 9     | 461.78   | 370.65     | 122.36   | 90.40    | 3.50 | 2.57                 | 0.37    | 0.17        |  |
| 0 g l <sup>-1</sup> | 100                          | 48.20   | 44.10      | 8        | 12    | 520.32   | 484.20     | 126.91   | 122.58   | 3.62 | 3.50                 | 0.42    | 0.21        |  |
|                     | 200                          | 56.20   | 52.30      | 11       | 14    | 631.97   | 523.57     | 154.14   | 127.70   | 4.40 | 3.64                 | 0.40    | 0.22        |  |
|                     | 300                          | 63.11   | 57.60      | 12       | 17    | 708.94   | 592.30     | 172.91   | 149.95   | 4.93 | 4.28                 | 0.49    | 0.28        |  |
| Mean value of       | of yeast 0 g l <sup>-1</sup> | 45.62   | 38.5       | 9        | 13    | 411.48   | 392.6      | 112.36   | 95.76    | 3.21 | 2.74                 | 0.47    | 0.28        |  |
| Yeast               | 0                            | 45.62   | 38.50      | 9        | 13    | 411.48   | 392.60     | 112.36   | 95.76    | 3.21 | 2.74                 | 0.47    | 0.28        |  |
| 2 g l <sup>-1</sup> | 100                          | 52.10   | 46.10      | 9        | 16    | 548.50   | 498.64     | 137.13   | 126.24   | 3.93 | 3.59                 | 0.49    | 0.27        |  |
|                     | 200                          | 58.90   | 53.20      | 14       | 16    | 652.30   | 613.54     | 159.10   | 149.64   | 4.55 | 4.28                 | 0.49    | 0.31        |  |
|                     | 300                          | 60.40   | 54.40      | 14       | 17    | 685.09   | 628.72     | 159.32   | 159.17   | 4.55 | 4.55                 | 0.50    | 0.30        |  |
| Mean value of       | of yeast 2 g l <sup>-1</sup> | 54.26   | 48.05      | 11.50    | 15.50 | 574.34   | 533.38     | 141.98   | 132.70   | 4.06 | 3.79                 | 0.49    | 0.29        |  |
| Yeast               | 0                            | 52.40   | 47.10      | 8        | 14    | 555.84   | 520.32     | 135.57   | 126.91   | 3.88 | 3.62                 | 0.49    | 0.30        |  |
| 4 g l <sup>-1</sup> | 100                          | 59.30   | 52.00      | 10       | 17    | 640.00   | 548.40     | 156.10   | 138.84   | 4.45 | 3.97                 | 0.51    | 0.31        |  |
|                     | 200                          | 65.30   | 60.10      | 16       | 20    | 718.69   | 675.33     | 171.12   | 164.72   | 4.88 | 4.71                 | 0.51    | 0.31        |  |
|                     | 300                          | 65.00   | 62.00      | 14       | 19    | 726.30   | 723.00     | 177.15   | 183.04   | 5.07 | 5.24                 | 0.49    | 0.30        |  |
| Mean value of       | of yeast 4 g l <sup>-1</sup> | 60.50   | 55.30      | 12.00    | 17.50 | 660.21   | 616.76     | 159.99   | 153.38   | 4.57 | 4.39                 | 0.50    | 0.31        |  |
| Yeast               | 0                            | 59.40   | 54.60      | 13       | 18    | 632.60   | 567.30     | 154.29   | 138.37   | 4.40 | 3.95                 | 0.51    | 0.32        |  |
| 6 g l <sup>-1</sup> | 100                          | 62.10   | 57.30      | 11       | 19    | 699.18   | 632.10     | 170.53   | 160.03   | 4.88 | 4.57                 | 0.51    | 0.32        |  |
|                     | 200                          | 69.32   | 64.20      | 18       | 22    | 758.80   | 718.50     | 185.07   | 175.24   | 5.28 | 5.00                 | 0.51    | 0.31        |  |
|                     | 300                          | 69.12   | 65.00      | 16       | 24    | 777.00   | 747.96     | 189.51   | 189.36   | 5.40 | 5.40                 | 0.51    | 0.32        |  |
| Mean value of       | of yeast 6 g l <sup>-1</sup> | 64.99   | 60.28      | 14.50    | 20.75 | 716.90   | 666.47     | 174.85   | 165.75   | 4.99 | 4.73                 | 0.51    | 0.32        |  |
| Mean value of       | of GA <sub>3</sub> at: 0     | 48.91   | 43.27      | 8.75     | 13.50 | 515.43   | 462.72     | 131.15   | 112.86   | 3.75 | 3.22                 | 0.46    | 0.27        |  |
| 100                 |                              | 55.43   | 49.88      | 9.50     | 16.00 | 602.00   | 540.84     | 147.67   | 136.92   | 4.22 | 3.91                 | 0.48    | 0.28        |  |
| 200                 |                              | 62.43   | 57.45      | 14.75    | 18.00 | 690.44   | 632.74     | 167.36   | 154.33   | 4.78 | 4.41                 | 0.48    | 0.29        |  |
| 300                 |                              | 64.41   | 59.75      | 14.00    | 19.25 | 724.33   | 673.00     | 174.72   | 170.38   | 4.99 | 4.87                 | 0.50    | 0.30        |  |
| LSD (5%)            | GA <sub>3</sub>              | 1.82    | 1.72       | 1.2      | 1.0   | 13.6     | 14.5       | 7.4      | 8.5      | 0.33 | 0.31                 | 0.05    | 0.04        |  |
|                     | Yeast                        | 2.45    | 2.14       | 0.8      | 0.85  | 12.2     | 10.67      | 6.8      | 9.3      | 0.19 | 0.14                 | 0.03    | 0.02        |  |
|                     | GA <sub>3</sub> x Yeast      | 3.1     | 2.94       | 1.3      | 1.1   | 22.3     | 24.58      | 12.6     | 14.1     | 0.45 | 0.33                 | 0.085   | 0.06        |  |

\*I - first harvest, \*\*II - second harvest.

ppm GA<sub>3</sub>), T6 (2 g  $\Gamma^1$  yeast + 100 ppm GA<sub>3</sub>), T7 (2 g  $\Gamma^1$  yeast + 200 ppm GA<sub>3</sub>), T8 (2 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>), T9 (4 g  $\Gamma^1$  yeast + 0 ppm GA<sub>3</sub>), T10 (4 g  $\Gamma^1$  yeast + 100 ppm GA<sub>3</sub>), T11 (4 g  $\Gamma^1$  yeast + 200 ppm GA<sub>3</sub>), T12 (4 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>), T13 (6 g  $\Gamma^1$  yeast + 0 ppm GA<sub>3</sub>), T14 (6 g  $\Gamma^1$  yeast + 100 ppm GA<sub>3</sub>), T13 (6 g  $\Gamma^1$  yeast + 200 ppm GA<sub>3</sub>), T14 (6 g  $\Gamma^1$  yeast + 100 ppm GA<sub>3</sub>), T15 (6 g  $\Gamma^1$  yeast + 200 ppm GA<sub>3</sub>), and T16 (6 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>). The experimental design was a completely randomized block with three replications for each treatment. Seeds of lemon balm were sown in a nursery on the 15<sup>th</sup> of November during the two seasons under plastic low-tunnels. The 15-20 cm seedlings were transplanted into the field in early April, 40 cm apart in rows 50 cm wide. The area of each plot was 4 m<sup>2</sup> with three rows contained a total of 15 plants. The plants were sprayed twice with GA<sub>3</sub>: the first spray four weeks after transplanting to the field; the second in July, 2 weeks after the first harvest. The foliar application of yeast was applied to leaves two weeks after the GA<sub>3</sub> spraying.

The following data were recorded for the two harvests (July and October): plant height (cm), number of branches, fresh and dry weight of plant (g/plant or kg/ha), EO yield per plant, and EO constituents.

Growth, yield, and chemical composition during the two seasons were statistically analyzed according to the procedure of Snedecor and Cochran (1980). The means of the treatments were compared using the least significant difference test at the 0.05 level. Hundred gram fresh weight of *M. officinalis* leaves were subjected to hydrodistillation using the method of Guenther (1961) and analyzed by GC-MS. The identification of volatile oil chromatogram's peaks was by comparing their mass spectra and retention times with those of standard *Melissa* EO, library and Adams (1989). The isolated EO was dehydrated over anhydrous sodium sulphate and stored in a refrigerator until gas chromatography (GC) analysis. The gas chromatograph (Perkin Elmer Model 3920B) was equipped with a thermal conductivity detector and a 2 m  $\times$  3 mm column packed with 10% Carbowax 20Mon 80/100 Chromsorb WAW, and hydrogen was used as the carrier gas at 0.5 cm<sup>3</sup> s<sup>-1</sup>. Temperature of injector, column and detector was 200, 130 and 200°C, respectively.

#### **RESULTS AND DISCUSSION**

Spraying lemon balm plants with different concentrations of GA<sub>3</sub> amended with 6 g  $\Gamma^1$  yeast increased plant height, number of branches, plant fresh and dry weight, and EO percentage compared with the control sprayed with water (**Table 3**). The largest values of growth and EO yield occurred with T16 (6 g  $\Gamma^1$  yeast + 300 ppm GA<sub>3</sub>), but the lowest values of growth and EO yield were observed in the control. The same trends were observed in the second season, but with slight differences in values as shown in **Table 4**. The stimulating effect of GA<sub>3</sub> on plant height is due to its effect on cell elongation. A similar effect of GA<sub>3</sub> on enhancing plant height was obtained by Foury (1977) on globe artichoke (*Cynara scolymus* L.). In addition, the enhancing effect of yeast on growth and EO yield of lemon balm plants may be due to its high content of minerals, in particular N, P and K, and also the high content of vitamin B, which plays an important role in improving plant growth

Table 4 Effect of GA<sub>3</sub> and dry yeast on growth, yield and essential oil percentage of lemon balm during the second season.

| Gibberellic                 | Plant height (cm)   |  | No. of branches  |  | Plant fresh   |  | Plant dry weight  |  | Plant dry weight   |  | Essential oil (%   |   |  |
|-----------------------------|---|--|--|--|---|--|---|--|--|--|--|---|--|
| acid (GA <sub>3</sub>       |   |  |  |  | weight (  | weight (g)   |   | (g)  |  | ha <sup>-1</sup> (t)   |  | dry weight)   |  |
| in ppm)                     | I*  | II**   | I*   | II**   | I*  | П**  | I*  | II**   | I*   | II**   | I*   | II**  |  |
| 0                           | 42.60   | 34.10  | 6  | 10   | 542.40  | 381.20   | 135.60  | 92.98  | 3.88   | 2.67   | 0.41   | 0.19  |  |
| 100                         | 48.00   | 42.00  | 7  | 12   | 561.53  | 513.77   | 136.96  | 125.31   | 3.90   | 3.57   | 0.46   | 0.23  |  |
| 200                         | 58.30   | 48.30  | 11   | 15   | 658.70  | 605.30   | 166.76  | 147.63   | 4.76   | 4.21   | 0.44   | 0.26  |  |
| 300                         | 65.40   | 54.30  | 12   | 16   | 698.30  | 671.04   | 174.58  | 169.88   | 4.97   | 4.86   | 0.47   | 0.32  |  |
| f yeast 0 g l <sup>-1</sup> | 53.58   | 44.68  | 9.00   | 13.25  | 615.23  | 542.83   | 153.48  | 133.95   | 4.38   | 3.83   | 0.45   | 0.25  |  |
| 0                           | 47.00   | 36.00  | 9  | 12   | 531.47  | 442.00   | 132.87  | 107.80   | 3.78   | 3.07   | 0.49   | 0.29  |  |
| 100                         | 50.60   | 46.00  | 10   | 17   | 615.30  | 532.10   | 150.07  | 129.78   | 4.28   | 3.71   | 0.53   | 0.31  |  |
| 200                         | 59.30   | 56.60  | 12   | 16   | 686.19  | 619.78   | 173.72  | 154.95   | 4.95   | 4.43   | 0.53   | 0.33  |  |
| 300                         | 63.20   | 58.00  | 14   | 18   | 703.66  | 633.76   | 175.92  | 160.45   | 5.02   | 4.59   | 0.49   | 0.33  |  |
| f yeast 2 g l <sup>-1</sup> | 55.03   | 49.15  | 11.25  | 15.75  | 634.16  | 556.91   | 158.15  | 138.25   | 4.51   | 3.95   | 0.51   | 0.32  |  |
| 0                           | 51.00   | 48.00  | 9  | 13   | 614.10  | 543.00   | 153.53  | 132.44   | 4.38   | 3.78   | 0.51   | 0.34  |  |
| 100                         | 62.30   | 50.00  | 10   | 17   | 690.85  | 605.80   | 168.50  | 147.76   | 4.81   | 4.21   | 0.50   | 0.37  |  |
| 200                         | 66.30   | 62.30  | 12   | 20   | 760.75  | 700.20   | 192.59  | 170.78   | 5.50   | 4.88   | 0.52   | 0.35  |  |
| 300                         | 68.40   | 65.87  | 13   | 17   | 750.30  | 718.00   | 187.58  | 181.77   | 5.36   | 5.19   | 0.47   | 0.33  |  |
| f yeast 4 g l <sup>-1</sup> | 62.00   | 56.54  | 11.00  | 16.75  | 704.00  | 641.75   | 175.55  | 158.19   | 5.01   | 4.52   | 0.50   | 0.35  |  |
| 0                           | 58.60   | 52.30  | 12   | 19   | 695.10  | 636.09   | 173.78  | 155.14   | 4.97   | 4.43   | 0.52   | 0.35  |  |
| 100                         | 64.50   | 58.00  | 12   | 19   | 726.40  | 662.90   | 177.17  | 161.68   | 5.07   | 4.62   | 0.54   | 0.37  |  |
| 200                         | 70.00   | 66.00  | 17   | 22   | 802.00  | 747.93   | 203.04  | 182.42   | 5.81   | 5.21   | 0.50   | 0.34  |  |
| 300                         | 72.00   | 69.00  | 14   | 22   | 800.30  | 757.50   | 200.08  | 191.77   | 5.71   | 5.47   | 0.48   | 0.34  |  |
| f yeast 6 g l <sup>-1</sup> | 66.28   | 61.33  | 13.75  | 20.50  | 755.95  | 701.11   | 188.52  | 172.75   | 5.39   | 4.93   | 0.51   | 0.35  |  |
| f GA <sub>3</sub> at: 0     | 49.80   | 42.60  | 9.00   | 13.50  | 595.77  | 500.57   | 148.945   | 122.09   | 4.25   | 3.49   | 0.48   | 0.29  |  |
|                             | 56.35   | 49.00  | 9.75   | 16.25  | 648.52  | 578.64   | 158.18  | 141.13   | 4.52   | 4.03   | 0.51   | 0.32  |  |
|                             | 63.48   | 58.30  | 13.00  | 18.25  | 726.91  | 668.30   | 184.03  | 163.95   | 5.26   | 4.68   | 0.50   | 0.32  |  |
|                             | 67.25   | 61.79  | 13.25  | 18.25  | 738.14  | 695.08   | 184.54  | 175.97   | 5.27   | 5.03   | 0.48   | 0.33  |  |
| GA <sub>3</sub>             | 1.77  | 1.85   | 0.9  | 1.1  | 10.6  | 12.3   | 8.2   | 9.1  | 0.29   | 0.33   | 0.04   | 0.05  |  |
| Yeast                       | 2.36  | 2.47   | 0.7  | 0.8  | 9.80  | 11.24  | 7.1   | 6.4  | 0.21   | 0.14   | 0.02   | 0.02  |  |
| GA <sub>3</sub> x Yeast     | 3.58  | 3.94   | 1.0  | 1.4  | 19.25   | 21.45  | 12.3  | 13.58  | 0.43   | 0.40   | 0.03   | 0.08  |  |
| 1                           | $\begin{array}{c} \mbox{acid} (GA_3 \\ \mbox{in ppm}) \\ 0 \\ 100 \\ 200 \\ 300 \\ f \ yeast \ 0 \ g \ l^{-1} \\ 0 \\ 100 \\ 200 \\ 300 \\ f \ yeast \ 2 \ g \ l^{-1} \\ 0 \\ 100 \\ 200 \\ 300 \\ f \ yeast \ 4 \ g \ l^{-1} \\ 0 \\ 100 \\ 200 \\ 300 \\ f \ yeast \ 6 \ g \ l^{-1} \\ f \ GA_3 \ at: \ 0 \\ \end{array}$ | acid (GA <sub>3</sub> in ppm)         I*           0         42.60           100         48.00           200         58.30           300         65.40           f yeast 0 g I <sup>-1</sup> 53.58           0         47.00           100         50.60           200         59.30           300         63.20           f yeast 2 g I <sup>-1</sup> 55.03           0         51.00           100         62.30           200         66.30           300         68.40           f yeast 4 g I <sup>-1</sup> 62.00           0         58.60           100         64.50           200         70.00           300         64.50           200         70.00           300         72.00           f yeast 6 g I <sup>-1</sup> 66.28           f GA <sub>3</sub> at: 0         49.80           56.35         63.48           67.25         6A <sub>3</sub> GA <sub>3</sub> 1.77           Yeast         2.36 | acid (GA <sub>3</sub> I*         II**           0         42.60         34.10           100         48.00         42.00           200         58.30         48.30           300         65.40         54.30           f yeast 0 g $I^{-1}$ 53.58         44.68           0         47.00         36.00           100         50.60         46.00           200         59.30         56.60           300         63.20         58.00           100         51.00         48.00           100         62.30         50.00           200         66.30         62.30           300         68.40         65.87           f yeast 4 g $I^{-1}$ 62.00         56.54           0         58.60         52.30           100         64.50         58.00           200         70.00         66.00           300         72.00         69.00           f yeast 6 g $\Gamma^{1}$ 66.28         61.33           f GA <sub>3</sub> at: 0         49.80         42.60           56.35         49.00         63.48         58.30           67.25         61.79         6 | acid (GA <sub>3</sub> I*         II*         I           0         42.60         34.10         6           100         48.00         42.00         7           200         58.30         48.30         11           300         65.40         54.30         12           f yeast 0 g I <sup>-1</sup> 53.58         44.68         9.00           0         47.00         36.00         9           100         50.60         46.00         10           200         59.30         56.60         12           300         63.20         58.00         14           f yeast 2 g I <sup>-1</sup> 55.03         49.15         11.25           0         51.00         48.00         9           100         62.30         50.00         10           200         66.30         62.30         12           300         68.40         65.87         13           f yeast 4 g I <sup>-1</sup> 62.00         56.54         11.00           0         58.60         52.30         12           100         64.50         58.00         12           200         70.00         66.00         17 | acid (GA3in ppm)I*II**I*II**042.6034.1061010048.0042.0071220058.3048.30111530065.4054.301216f yeast 0 g I <sup>-1</sup> 53.5844.689.0013.25047.0036.0091210050.6046.00101720059.3056.60121630063.2058.001418f yeast 2 g I <sup>-1</sup> 55.0349.1511.2515.75051.0048.0091310062.3050.00101720066.3062.30122030068.4065.871317f yeast 4 g I <sup>-1</sup> 62.0056.5411.0016.75058.6052.30121910064.5058.00121920070.0066.00172230072.0069.001422f yeast 6 g I <sup>-1</sup> 66.2861.3313.7520.50f GA <sub>3</sub> at: 049.8042.609.0013.5056.3549.009.7516.2563.4858.3013.0018.256A <sub>3</sub> 1.771.850.91.1Yeast2.362.470.70.8 | weight (in 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<math>\Gamma^1</math>62.0056.5411.0016.75704.00641.75175.55058.6052.301219726.40662.90177.1720070.0066.001722800.30757.50200.0</td> <td>weight (g)(g)in ppm)I*II*II*II*II*II042.6034.10610542.40381.20135.6092.9810048.0042.00712561.53513.77136.96125.3120058.3048.301115658.70605.30166.76147.6330065.4054.301216698.30671.04174.58169.88fyeast 0 g I<sup>-1</sup>53.5844.689.0013.25615.23542.83153.48133.95047.0036.00912531.47442.00132.87107.8010050.6046.001017615.30532.10150.07129.7820059.3056.601216686.19619.78173.72154.9530063.2058.001418703.66633.76175.92160.45f yeast 2 g I<sup>-1</sup>55.0349.1511.2515.75634.16556.91158.15138.25051.0048.00913614.10543.00153.53132.4410062.3050.001017690.85605.80168.50147.7620066.3062.301219760.75700.20192.59170.7830068.4065.871317750.30718.00187.58181.77f yeast 4 g I<sup>-1</sup><td>weight (g)(g)ha<sup>-1</sup> (f)in ppm)I*II*<th< td=""><td>weight (g)(g)ha<sup>1</sup> (f)in 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(g)(g)har<sup>1</sup> (t)dry weight (g)in ppm)I*II**I*II**I*II**I*II**I*II**I*II**I*II**I*II*</td></th<></td> | weight (g)(g)ha <sup>-1</sup> (f)in ppm)I*II* <th< td=""><td>weight (g)(g)ha<sup>1</sup> (f)in ppm)I*II*I*II*II*II*IIIIIIIII042.6034.10610542.40381.20135.6092.983.882.6710048.0042.00712561.53513.77136.96125.313.903.5720058.3048.301115658.70605.30166.76147.634.764.2130065.4054.301216698.30671.04174.58169.884.974.86f yeast 0 g I<sup>-1</sup>53.5844.689.0013.25615.23542.83153.48133.954.383.83047.0036.00912531.47442.00132.87107.803.783.0710050.6046.001017615.30532.10150.07129.784.283.7120059.3056.601216686.19619.78173.72154.954.954.4330063.2058.001418703.66633.76175.92160.455.024.59f yeast 2 g I<sup>-1</sup>55.0349.1511.2515.75634.16556.91158.15138.254.513.95051.0048.00913614.10543.00153.53132.444.383.7810062.3050.001017690.85</td><td>weight (g)(g)har<sup>1</sup> (t)dry weight (g)in ppm)I*II**I*II**I*II**I*II**I*II**I*II**I*II**I*II*</td></th<> | weight (g)(g)ha <sup>1</sup> 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Table 5 Chemical composition of lemon balm essential oil as affected by yeast and/or GA<sub>3</sub> treatments.

| Compounds                                  | Relative concentration of compounds (%) |       |          |       |           |       |       |       |  |  |  |
|--|---|-------|----------|-------|-----------|-------|-------|-------|--|--|--|
|  | Control                                 | T4    | T13      | T16   | Control   | T4    | T13   | T16   |  |  |  |
|  |   | Ha    | arvest 1 |       | Harvest 2 |       |       |       |  |  |  |
| Camphene                                   | 0.13                                    | 0.12  | 0.13     | 0.10  | 0.13      | 0.13  | 0.14  | 0.09  |  |  |  |
| β-pinene                                   | 0.09                                    | 0.07  | 0.08     | 0.04  | 0.09      | 0.12  | 0.04  | 0.23  |  |  |  |
| β-phellandrene                             | 0.05                                    | 0.06  | 0.03     | 0.05  | 0.06      | 0.07  | 0.10  | 0.09  |  |  |  |
| Limonen                                    | 0.74                                    | 0.88  | 0.81     | 0.63  | 0.32      | 1.14  | 1.12  | 0.85  |  |  |  |
| α-terpinene                                | 0.40                                    | 0.64  | 0.44     | 0.64  | 0.42      | 0.24  | 0.34  | 0.63  |  |  |  |
| cymene                                     | 0.82                                    | 1.14  | 1.12     | 1.23  | 1.16      | 0.85  | 1.20  | 0.95  |  |  |  |
| 6-methyl-5-heptene-2-ol                    | 3.82                                    | 3.88  | 4.12     | 4.16  | 3.24      | 5.16  | 5.23  | 6.17  |  |  |  |
| linalool                                   | 0.82                                    | 1.14  | 1.01     | 0.93  | 0.65      | 0.93  | 0.84  | 1.32  |  |  |  |
| citronellal                                | 13.35                                   | 16.38 | 14.26    | 16.21 | 11.26     | 13.65 | 14.65 | 10.21 |  |  |  |
| β-caryophyllene                            | 4.85                                    | 4.63  | 4.31     | 5.81  | 5.65      | 7.16  | 5.84  | 8.35  |  |  |  |
| neral                                      | 18.74                                   | 17.45 | 12.10    | 18.21 | 26.34     | 28.33 | 22.14 | 15.64 |  |  |  |
| geranial                                   | 26.84                                   | 28.17 | 30.18    | 32.12 | 24.15     | 17.60 | 26.13 | 29.16 |  |  |  |
| geranyl acetate                            | 1.65                                    | 0.87  | 0.97     | 1.15  | 1.99      | 0.73  | 0.84  | 0.94  |  |  |  |
| neryl acetate                              | 1.42                                    | 2.10  | 1.82     | 1.82  | 1.76      | 0.94  | 0.82  | 0.75  |  |  |  |
| geraniol                                   | 4.23                                    | 1.86  | 4.34     | 2.99  | 6.14      | 2.10  | 4.16  | 4.21  |  |  |  |
| nerol                                      | 0.64                                    | 0.75  | 1.65     | 0.13  | 0.35      | 0.21  | 0.42  | 0.14  |  |  |  |
| β-caryophyllene oxide                      | 9.94                                    | 7.43  | 8.12     | 4.13  | 6.30      | 5.17  | 7.35  | 8.16  |  |  |  |
| Total of identified compounds              | 88.51                                   | 87.57 | 85.50    | 90.36 | 90.02     | 84.53 | 91.37 | 87.91 |  |  |  |
| Total of unidentified compounds (unknowns) | 11.49                                   | 12.43 | 14.51    | 9.64  | 9.98      | 15.47 | 8.63  | 12.09 |  |  |  |
| Total                                      | 100                                     | 100   | 100      | 100   | 100       | 100   | 100   | 100   |  |  |  |

Control - 0 g l<sup>-1</sup> yeast + 0 ppm GA<sub>3</sub>, sprayed with water, T4 - 0 g l<sup>-1</sup> yeast + 300 ppm GA<sub>3</sub>, T13 - 6 g l<sup>-1</sup> yeast + 0 ppm GA<sub>3</sub>, T16 - 6 g l<sup>-1</sup> yeast + 300 ppm GA<sub>3</sub>

(Subba 1984). Furthermore, vitamin B affects meristemic tissue, plant growth and development indirectly by stimulating the endogenous levels of different plant hormones such as cytokinins and gibberellins (Kodendaramaiah and Gopala Rao 1985).

These results are in agreement with those of El-Khateeb (1989) and Mohamed and Wahba (1993) for rosemary (Rosmarinus officinalis), Turky (1989) for coriander (Coriandrum sativum), Shedeed et al. (1990) and Bedour et al. (1995) for basil (Ocimum basilicum), and Abou-Taleb et al. (1998) for thyme (Thymus vulgaris), all of whom reported that foliar spray of GA<sub>3</sub> resulted in the highest values of vegetative growth including plant height and fresh and dry weight of the investigated plants, using GA<sub>3</sub> at a range of

50-400 ppm. Mousa (1994) found that GA<sub>3</sub> at 200 ppm increased the plant height, and fresh and dry weight, but that GA<sub>3</sub> treatments (50, 100, 150 or 200 ppm) had no significant effect on the number of branches/plant; however, they significantly increased the total number and fresh and dry weight of the flowering heads of marigold (Calendula officinalis) and rue (Ruta graveolens) plants.

The highest EO percentage (0.32 and 0.37%) was obtained by spraying the yeast at 6 g  $\Gamma^1$  while the lowest EO percentage (0.17 and 0.19%) resulted with the control treat-ment (0 g  $l^{-1}$  yeast + 0 ppm GA<sub>3</sub>), as shown in **Tables 3** and 4. All treatments altered the composition of EO in lemon balm plants. These results agree with those of Liang et al. (1996), who studied the effect of foliar application of  $GA_3$  on chemical composition of leaves of the tea plant (*Camellia sinensis*). They found that the concentration of amino acids increased by 9.8% while the concentration of polyphenols and the polyphenol: amino acids ratio decreased by 9.9 and 11.5%, respectively. The response to gibberellins differed between clones and with shoot development stage. Weathers *et al.* (1996) found that the addition of GA<sub>3</sub> (0.001-0.01 mg<sup>-1</sup>) to *Artemisia annua* increased the biomass yield. Artemisinin production was best with GA<sub>3</sub> (0.01 mg<sup>-1</sup>).

Values in **Table 5** are of lemon balm EO analysis of the first season only; values of the second season showed a similar trend with slight differences in values. The analysis of the EO in lemon balm (Table 5) showed the presence of 17 compounds. The major compound was geranial, followed by neral. The minor compounds were  $\alpha$ -pinene and  $\beta$ -phellandrene. Fresh weight of 100 g of *M. officinalis* leaves of different treatments were subjected to hydrodistillation and analyzed by GC-MS. The identification of the peaks in the volatile oil's chromatogram was through the comparison of their mass spectra with those of standard Melissa EO, library and Adams (1989), listed in Table 5. Total identified compounds percentage were 88.513, 87.57, 85.495, 90.361, 90.02, 84.53, 91.37 and 87.91 for control, T4 (0 g  $l^{-1}$  yeast + 300 ppm GA<sub>3</sub>), T13 (6 g  $l^{-1}$  yeast + 0 ppm GA<sub>3</sub>) and T16 (6 g  $l^1$  yeast + 300 ppm GA<sub>3</sub>) for the first and second harvests, respectively.

The major amount of volatile principle was oxygenated compounds which constituted 81.45, 80.03, 78.57, 81.85, 82.18, 74.82, 82.58 and 76.71% of total volatile constituents for control, T4, T13 and T16 for the first and second harvests, respectively. The major oxygenated compound is geranial (26.84% for control group) followed by neral (18.74%), citronellal (13.35%),  $\beta$ -caryophyllene oxide (9.94%), geraniol (4.23%) and 6-methoxy-5-heptene-2-ol (3.82%).

The oxygenated compounds constituted oxide form as β-caryophyllene oxide (9.94, 7.433, 8.120, 4.130, 6.300, 5.170, 7.350 and 8.160% for control, T4, T13 and T16, for the first and second harvests, respectively, all treatments decreased the  $\beta$ -caryophyllene oxide percentage compared to the control group. The percentage alcohol was 9.51, 7.63, 11.12, 8.21, 10.38, 8.4, 10.65 and 11.84% for control, T4, T13 and T16, for the first and second harvests, respectively, the  $GA_3$  treatment decreased the percentage alcohol (7.63) and 8.4%; first and second harvests, respectively), which was increased by the application of yeast in the first and second harvests (11.12 and 10.65%) as compared to control group. The percentage aldehydes was 58.93, 62, 56.54, 66.54, 61.75 59.58, 62.92 and 55.01% for control, T4, T13 and T16, for the first and second harvests, respectively; all treatments increased the aldehyde percentage compared to the control group, GA<sub>3</sub> being the most effective. The acetate derivatives were 3.07, 2.97, 2.79, 2.97, 3.75, 1.67, 1.66 and 1.69% for control, T4, T13 and T16 for the first and second harvests, respectively. Values of the first harvest show that, the major alcohol was geraniol (4.23%) followed by heptene-2-ol (3.82%), while aldehydes were the main compounds of lemon balm volatile oil (58.93%). The aldehyde compounds are composed of geranial (26.84% for control group), neral (18.74%) and citronellal (13.35%). The acetate derivatives are geranyl acetate (1.65%) and neryl acetate (1.42%) while the only oxide compound detected was  $\beta$ -caryophyllene oxide (9.94%). In conclusion GA<sub>3</sub> foliar application is superior to all other treatments to increase the percentage of oxygenated compounds, especially aldehyde compounds, while the application of yeast is the best treatment to increase the percentage alcohol.

The amount of volatile hydrocarbons are 7.07, 7.54, 6.92, 8.51, 7.84, 9.71, 8.78 and 11.2% for control, T4, T13 and T16 for the first and second harvests, respectively. The major hydrocarbons are  $\beta$ -caryophyllene (4.85%) followed by cymene (0.82%) then limonene (0.74%). The hydrocarbon percentage was increased by application of yeast while it decreased by application of GA<sub>3</sub>.

## CONCLUSION

Using 6 g  $l^{-1}$  active dry yeast + 300 ppm GA<sub>3</sub> resulted in the best lemon balm plant growth and yield.

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